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ASSESSMENT OF MICROBIAL QUALITY AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF SOME LIQUID HERBAL REMEDIES SOLD IN OWERRI METROPOLIS.

Udensi, J.U[1] ., Anyanwu, C.O[2] ., Akanazu, C.O[3]., Onyima, E.C[1]., Okafor, S.O[2] & Udokwu, K.C[1].

1 Environmental Health Science Department, Federal University of Technology, P.M.B. 1526, Owerri, Imo State,Nigeria.

2 Department of Biotechnology, Federal University of Technology, P.M.B. 1526, Owerri, Imo State, Nigeria.

3 Department of Public Health, Federal University of Technology, P.M.B. 1526, Owerri, Imo State, Nigeria.

Corresponding Author's Email: Udensiugochi@gmail.com

Abstract

Assessment of microbial quality and antimicrobial susceptibility pattern of some liquid herbal medicines sold within Owerri metropolis was investigated. The assessment of microbial contamination on the herbal products (A-E) was carried out using standard laboratory methods. Spread plate technique was used to cultivate serially diluted portions of the samples. Results obtained from this study revealed the presence of microbial contaminants. The total heterotrophic bacterial count of the various samples showed that sample A had the highest growth of 2.46 x 10^5cfu/ml and least growth of 7.6 x 10^5cfu/ml was recorded for sample D. Samples B and C had growth of 2.04 x 10^5 and 9.8 x 10^5cfu/ml respectively. Sample E recorded no growth. Total heterotrophic fungal count was revealed that sample A had 5.8 x 10^5cfu/ml while sample B had 4.3 x 10^5cfu/ml. No growth was recorded for the remaining samples. One way analysis of variance (ANOVA) showed no significant difference ($p<0.05$) in the microbial load of the herbal medicines. A total of twelve isolates were obtained from this study, eleven bacteria and a fungus (*Candida sp.*) were identified. *E.coli* and *S. aureus* represent 27.27% of the isolates while *Klebsiella* sp. and *Proteus* sp. represent 18.18%. The least percentage occurrence was 9.09% for *Psuedomonas* sp. Antibiotic susceptibility test showed that the isolates were susceptible at the varying degrees. Zone of inhibition ranged from 11 mm to 30 mm. *E.coli* had the highest and lowest zones of inhibition of 30 mm and 11 mm respectively for sample. However, there was a statistical difference ($p>0.005$) in the antibiotic susceptibility pattern of bacteria isolates from the samples. The presence of microorganisms in herbal medicines is an indication of microbial contamination which could be hazardous to human health.

Keywords: Antimicrobial, Assessment, Microbial, Spread plate, Susceptibility.

1.0 INTRODUCTION

Plant is an important source of medicine and plays a key role in world health. Medicinal herbs or plants have been known to be an important potential source of therapeutics or curative aids. The use of medicinal plants has attained a commanding role in health system all over the world. This involves the use of medicinal plants not only for the treatment of diseases but also as potential material for maintaining good health and conditions (Oladeji, 2016).

Archibong *et al*., (2017) reported the term "herbal drugs" as plants and plant parts that have been converted into phytopharmaceuticals by means of some simple processes involving harvesting, drying and storage. According to the World Health Organization, herbal preparations contain plant parts or plant material in crude or processed state as active ingredients and may contain excipients (foreign substances) (WHO, 2003).

Nowadays it has been estimated that more than 50% of available drugs have originated in some way from plants (Heidarian and Rafieian-Kopaei, 2013). While medicinal plants were used primarily in simple pharmaceutical formulations such as macerations, infusions and decoctions, between the 16th and 18th centuries, the demand for compounded drugs was very much on the increase. These compounded drugs comprised medicinal plants along with drugs of animal and plant origin.

Furthermore, considerable efforts were invested in the study of optimal conditions for cultivating and manufacturing medicinal plants (Subhan *et al.,* 2010) since active components are normally more abundant from such well-maintained natural sources (Subhan *et al*., 2010). Currently, due to the potent side effects of modern synthetic drugs and increasing contraindications to their usage, a popular resurgence has materialized for the use of medicinal plants (Nasri and Shirzad, 2013).

In Nigeria, even though, there is proliferation of herbal products in the market, not so much has been done in this field. Some producers of herbal preparations in Nigeria do not have the required expertise to perform quality control on the preparation they produce. This brings about the problem of inconsistency on the quality of the herbal preparation in the country (Archibong *et al*., 2017).

The safety of herbal products is a major concern in public health (Kosalec and Tomic, 2009). This is because microorganisms of various kinds are naturally adherent to leaves, stem, flowers, seeds, and roots from which herbal medicine can be prepared and potential pathogens may also be introduced during harvesting, handling (Kosalec and Tomic, 2009: Danladi *et al*., 2009), open-air drying, preserving, manufacturing, (Kosalec and Tomic, 2009), and use of contaminated materials for storage (Danladi *et al*., 2009: Khattak, 2012). According to (Khattak, 2012: Esimone *et al*., 2007) some reports, the consumers may possibly fall into illness because of taking herbs incriminated with pathogenic microorganisms and sometimes the presence of antibiotic resistant microbial isolates in the herbal medicines will lead to transfer of antibiotic resistance strains to consumers.

It is therefore the aim of this study to assess the microbial quality and antibacterial susceptibility of some herbal medicines sold within Owerri metropolis.

2.0 MATERIALS AND METHODS

STUDY AREA

Owerri is the capital of Imo State in Nigeria (latitude: 5° 28' 34.7160" N, Longitude: 7° 1' 33.0708" E). It is also the state's largest city, followed by Orlu and Okigwe as second and third respectively. Owerri consists of three Local Government Areas including Owerri Municipal, Owerri North and Owerri West, it is approximately 100 square kilometres (40 sq mi) in area. Owerri is bordered by the Otamiri River to the east and the Nworie River to the south.

Source: Alex, 2008

Sample Collection

Five different samples of locally prepared herbal infusions was bought from three different locations and designated as A to E. The plant species origins of the herbal drugs were not ascertained, but the infusions were

classified based on the diseases cured as claimed by the vendors. Such diseases include malaria, Ulcer, worm, staphylococcal infection, typhoid fever and pile. The infusions were collected in sterile containers and transported to the laboratory for analyses. **Total Heterotrophic Bacteria (THB) Count**:

Aliquots of 1 ml of the sample was pipetted from 10^{-2} and 10^{-3} dilution tubes into well labeled Petri dishes. The plates were incubated for 24 hrs at 37°C. Then the colonies which developed on the plates were counted using a colony counter and expressed as colony forming unit per millilitre (cfu/ml). Each sample was analyzed in triplicates and the average was recorded. The colonies differing in size, shape and colour were selected from the different plates on nutrient agar and subcultured repeatedly to obtain pure isolates. The pure isolates were maintained on agar slant for further characterization and identification (Bala *et al*., 2017) **Total Heterotrophic fungal (THF) Count**:

The fungal count was determined by pipetting 1 ml of the serially diluted herbal infusion on Sabouraud Dextrose Agar (SDA) containing 0.01% chloramphenicol. The plates were incubated for 3 days at ambient temperature (Bala *et al*., 2017)

Characterization and Identification of isolates.

The characterization and identification of the bacterial isolates were carried out based on cell morphology, Gram's reaction and biochemical tests according to methods described by Oyeleke and Manga, (2008). The isolates were identified by comparing with those of known taxa using the schemes of Cowan and Steel (1973) as reported by (Bala *et al*., 2017).

Antibiotic Susceptibility Test

Bacteria isolated from the herbal preparations were screened for their susceptibilities to the selected antibiotics, clinical laboratory and standard institute (CLSI, 2005) methods were employed. Antimicrobial sensitivity test of bacteria was done using the disc diffusion method on Mueller Hinton Agar. Pure isolates were emulsified in a small volume of peptone water and the turbidity of the suspension was matched against McFarland standard (CLSI, 2005). About 0.1ml of the suspension was spread on the agar plate. Commercial antibiotic discs were aseptically placed on the inoculated plates and incubated at 35ºC for 24hours. The zones of inhibition (mm) were measured and recorded. The antibiotic discs and their concentration were Ampicillin (AMP, 10 µg), Cefepime (FET 30 µg), Cotrimazole (COT, 25 µg), Ceftriaxone (CRO, 30 µg), Ciprofloxacin (CPR 5 µg), Ceftazidimin (CAZ 30 µg), Ceftotaxime (CTX, 30 µg), Cefoxitin (FOX, 30 µg), Cefuroxime (CRX, 30 µg), Flucoxacillin (FLX, 5 µg), Erythromycin (ERY, 15 µg), Gentamicin (GEN, 10 µg), Lyncipro (LCP, 5 µg), Lyntriazone (LYN, 5 µg), Micromox (MCX, 5 µg), Penicillin (PEN, 10µ g), Nitrofurantoin (F, 300µ g) and Tetracycline (TET, 30 µg).

RESULTS AND DISCUSSION Total Heterotrophic Bacteria and Fungi Counts

The total heterotrophic bacterial and fungal counts from the different herbal infusions are shown in table 1: Sample A recorded the highest growth with 2.46x 10^5 cfu/ml while sample D had the least growth with 7.6 x 10^5 cfu/ml. Sample B and C had 2.04 x 10^5 cfu/ml and 9.8 x 10^5 cfu/ml respectively. No growth was recorded for sample E. The highest fungal count was obtained in sample A with 5.8 x 10^5 cfu/ml and least with 4.3 x 10^5 for sample B: samples D and E recorded no growth.

Table 1: Total Heterotrophic Bacteria and Fungi Counts

Sample Code	THBC (cfu/ ml)	THFC (cfu/ml)
A	2.46 x 10^5	5.8 x 10^5
B	2.04 x 10^5	4.3 x 10^5
C	9.8 x 10^5	No growth
D	7.6 x 10^5	No growth
E	No growth	No growth

Morphological and Biochemical Characteristics of Bacterial Isolates

Table 2 shows the morphological and chemical characteristics of bacterial isolates. All the isolates were catalase positive and all except isolate A4 were positive for methyl red: isolates A2, B4 and D are cocci while all the other isolates are rods. **Table 4.2: Morphological and Biochemical Characteristics of Bacterial Isolates.**

ISOLATE CODE	MICROSCOPIC CHARACTERISTICS	GRAM STAIN	INDOLE	MOTILITY	OXIDASE	CITRATE	UREASE	CATALASE	COAGULASE	METYL RED	MOST PROBABLE ORGANISM
SAMPLE A A1	Rod	-	+	+	-	-	-	+	0	+	*Escherichia coli*
A2	Cocci	+	_	_	_	+	+	+	+	+	*Staphylococcus sp*
A3	Rod	-	-	+	-	+	+	+	0	+	*Proteus sp*
A4	Rod	-	-	+	+	+	-	+	0	-	*Pseudomonas sp*
A5	Rod	-	-	-	-	+	+	+	0	+	*Klebsiella sp*
SAMPLE B B1	Rod	-	-	-	-	+	+	+	0	+	*Klebsiella sp*
B2	Rod	+	+	+	-	-	-	+	0	+	*Escherichia coli*
B3	Rod	-	-	+	-	+	+	+	0	+	*Proteus sp*
B4	Cocci	+	_	_	_	+	+	+	+	+	*Staphylococcus sp*
SAMPLE C1	Rod	-	+	+	-	-	-	+	0	+	*Escherichia coli*
SAMPLE D	Cocci	+	_	_	_	+	+	+	+	+	*Staphylococcus sp*

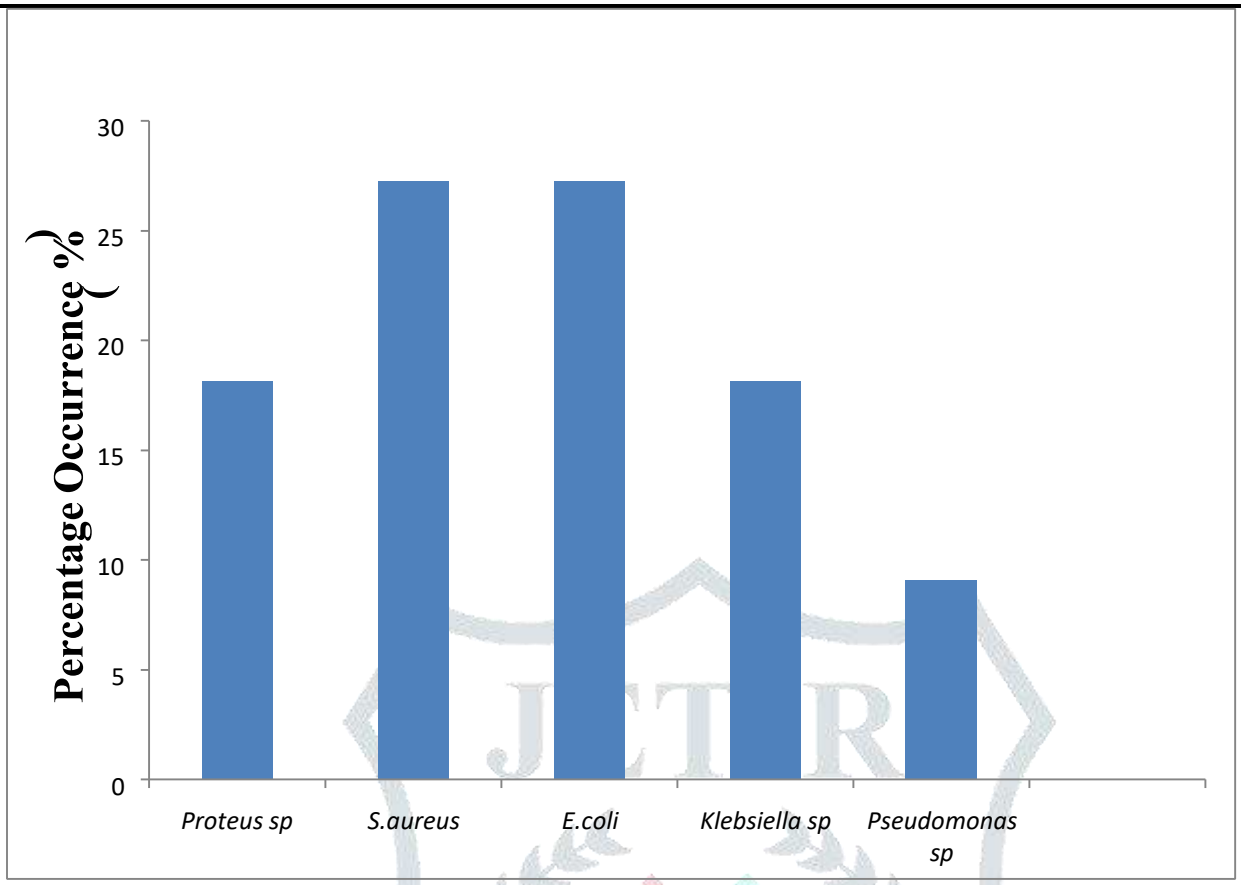

Fig 1. Percentage occurrence of bacterial isolates in the herbal medicines.

Antibiotic susceptibility test

Table 3: Antibiotic susceptibility test of five bacteria isolated on herbal medicinal products.

Antibiotics (µg)	Zone of inhibition (mm)				
	Proteus sp	*S.aureus*	*E.coli*	*Klebsiella sp*	*Pseudomonas sp*
CAZ	24S	23S	21S	24S	26S
CTX	24S	24S	24S	20R	-
CPR	20I	20I	30S	20I	27S
MCX	14R	15R	25S	14R	-
LYN	25S	25S	20I	25S	20I
F	-	-	17S	-	14R

CRX	13R	-	-	14R	-
GEN	-	-	16I	18S	-
FEP	24S	24S	-	24S	24S
FOX	18S	20I	11R	18S	21S
TET	-	-	-	14R	-
COT	-	-	-	15R	-

Note: R= Resistance, I= Intermediate and S= Sensitive.

COT=Cotrimazole (25µg), CPR=Ciprofloxacin (5µg), CAZ=Ceftazidimin (30µg), CTX=Ceftotaxime (30µg), FEP= Cefepime (30 µg), FOX=Cefoxitin (30µg), CRX=Cefuroxime (30µg), GEN=Gentamicin (10µg), LCP=Lyncipro (5µg), LYN= Lyntriazone (5µ g), MCX=Micromox (5µg), TET=Tetracycline (30µg).

DISCUSSION

Medicinal plants are mostly prepared in open environment and unhygienic condition which gradually lead to contamination of pathogens having public health importance. The samples were contaminated to varying degrees with bacteria and fungi which are in agreement with previous works on microbial quality by (Idu *et al*., 2011: Bala *et al*., 2017: Archibong *et al*., 2017). Statistical analysis showed a significant difference ($p<0.05$) in the microbial load of the herbal medicines indicating varying high level of microbial between the samples. However, one of the eleven (11) herbal infusion samples was however free from microbial contamination. The high counts of bacteria detected in the herbal infusion in this present study may be due to poor hygiene, use of contaminated water for washing and preparation, use of contaminated equipment and contaminated packaging materials. Other possible sources of contaminants are the personnel that could introduce the microbes when handling the raw materials during processing. Therefore, the process of harvesting, drying, storage, handling and the soil, influence the bacteriological quality of raw materials which in turn affect the entire quality of the herbal infusions.

The presence of *E.coli* in this study reflects the situation of faecal contamination is in corroboration with the findings of various authors (Archibong *et al*., 2017). This can be taken as an indicator for undesirable hygiene conditions. *E. coli* which had a percentage occurrence of

27.77 is a well-known enteropathogen and is the most common causative agent of childhood diarrhea of bacterial origin as reported by (Bonkoungou *et al*., 2013). The frequency of *E.coli* infection has led to concern over a demand for therapeutics to treat acute *E.coli* infections. *Staphylococcus aureus* which contaminated 27.27% of the samples has been associated with a number of complications especially to immuno-compromised individuals. However, contamination could provide amount of enterotoxin produced by some *Staphylococcus sp*, depending on the specific nature of the individual as noted by. Kosalec *et al*. (2009)

according to research, diarrheal episodes of infective aetiology represent around 27% of those reported, leading to a number of serious complications and high mortality rates (Archibong *et al*., 2017).

It can produce proteins that disable the immune system and damage tissues. It may also release exotoxins which cause gastroenteritis. The finding of other potential pathogens may also be significant. However, the high percentage occurrence of *E.coli* and *S.aureus* is in line with the findings of Bala *et al*., (2017) who recorded 28.6% for both *E.coli* and *S.aureus.*

Antibiotic susceptibility studies on the bacterial contamination of the liquid herbal products indicated that the isolates were susceptible to some of the antibiotics tested and resistant to others. *Proteus sp* responded positively (susceptibility) to ceftazidimin, ceftotaxime, lyntriazone, cefepime and cefoxitine while cefuroxime was highly resistant *to proteus sp*. However, ceftazidimin, ceftotaxime, lyntriazone, and cefepime were susceptible to *S.aureus* while micromox was resistant. Five out of the eight tested antibiotics were susceptible to E. coli and highest susceptibility was recorded for ciprofloxacin with 30 mm zone of inhibition. *Klebsiella sp* responded positively (susceptible) to five of the eleven tested antibiotics and highest zone of inhibition 25 mm was recorded for lyntriazone. Futhermore, *Pseudomonas sp* was susceptible to four of the six tested antibiotics with 27 mm for ciprofloxacin as the zone of inhibition. However, statistical analysis showed a significant difference ($p<0.05$) in the antibiotics susceptibility pattern of the isolates to herbal remedies. These antibiotics with high zones of inhibition are strongly recommended for the treatment of suspected cases of infections arising from the intake of contaminated herbal remedies.

Alwakeel (2008) reported that some environmental bacterial isolates are sometimes susceptible to antibiotics because resistance transfer is thought to be much less efficient in the environment where microorganisms are widely separated by distance as compared with human and animal intestines.

Good manufacturing practice (GMP) is strongly advocated to produce herbal remedies with wholesome quality.

Conclusion

The present study showed that herbal medicinal preparations sold in the study area were highly contaminated with pathogenic microorganisms with very high microbial load. Multiple drug resistance was not uncommon some of the isolates were resistant to 2 or more antibiotics tested.

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